THE MOLECULAR BIOLOGY OF BLADDER CANCER

DEMYSTIFYING MEDICINE

March 31, 2015

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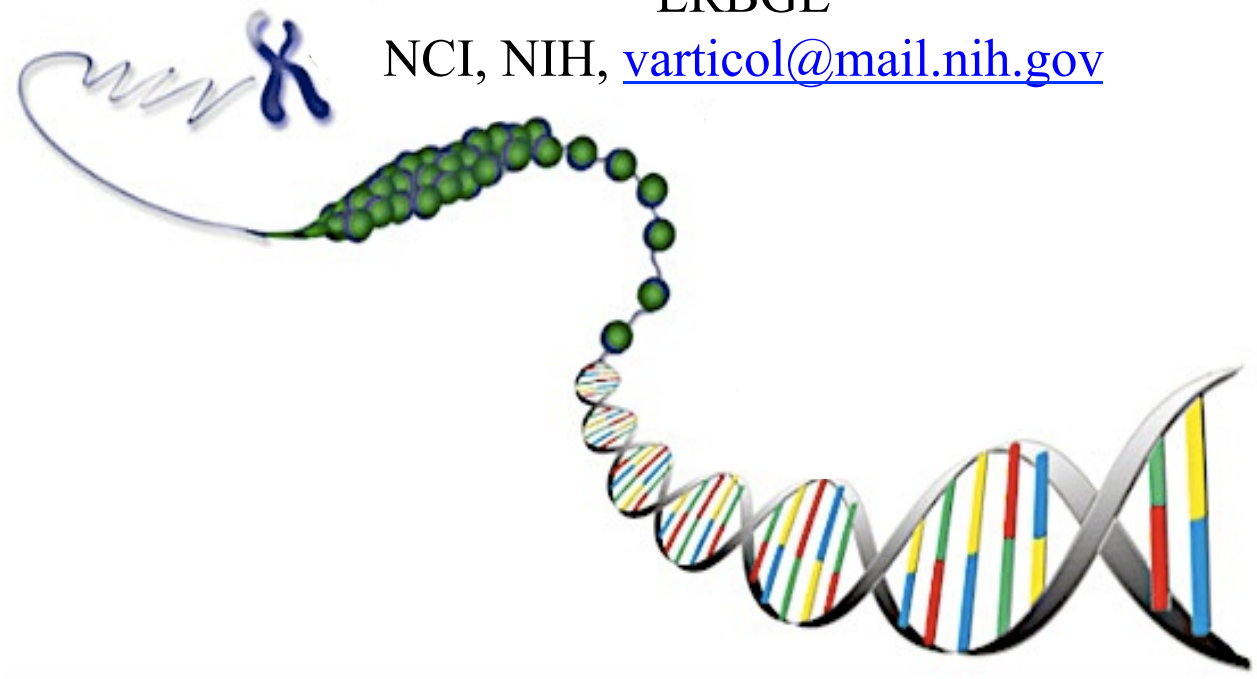


THE MOLECULAR BIOLOGY OF BLADDER CANCER

1) Analysis of molecular alterations in many tumor types
2) What is unique for Bladder Cancer (BLCA)
    a. Genomic alterations
    b. Epigenetics
    c. Chromatin modifications
    d. What is known
    e. What is new

3) Future trends

Genomic Landscape of Cancer

The Cancer Genome Atlas (TCGA) project, established in 2005, is a comprehensive and coordinated effort to accelerate our understanding of the molecular basis of cancer through the application of genomic technologies.

ARTICLE

OPEN

doi:10.1038/nature12634

Mutational landscape and significance across 12 major cancer types

Cyriac Kandoth[1]*, Michael D. McLellan[1]*, Fabio Vandin[2], Kai Ye[1,3], Beifang Niu[1], Charles Lu[1], Mingchao Xie[1], Qunyuan Zhang[1,3], Joshua F. McMichael[1], Matthew A. Wyczalkowski[1], Mark D. M. Leiserson[2], Christopher A. Miller[1], John S. Welch[4,5], Matthew J. Walter[4,5], Michael C. Wendl[1,3,6], Timothy J. Ley[1,3,4,5], Richard K. Wilson[1,3,5], Benjamin J. Raphael[2] & Li Ding[1,3,4,5]

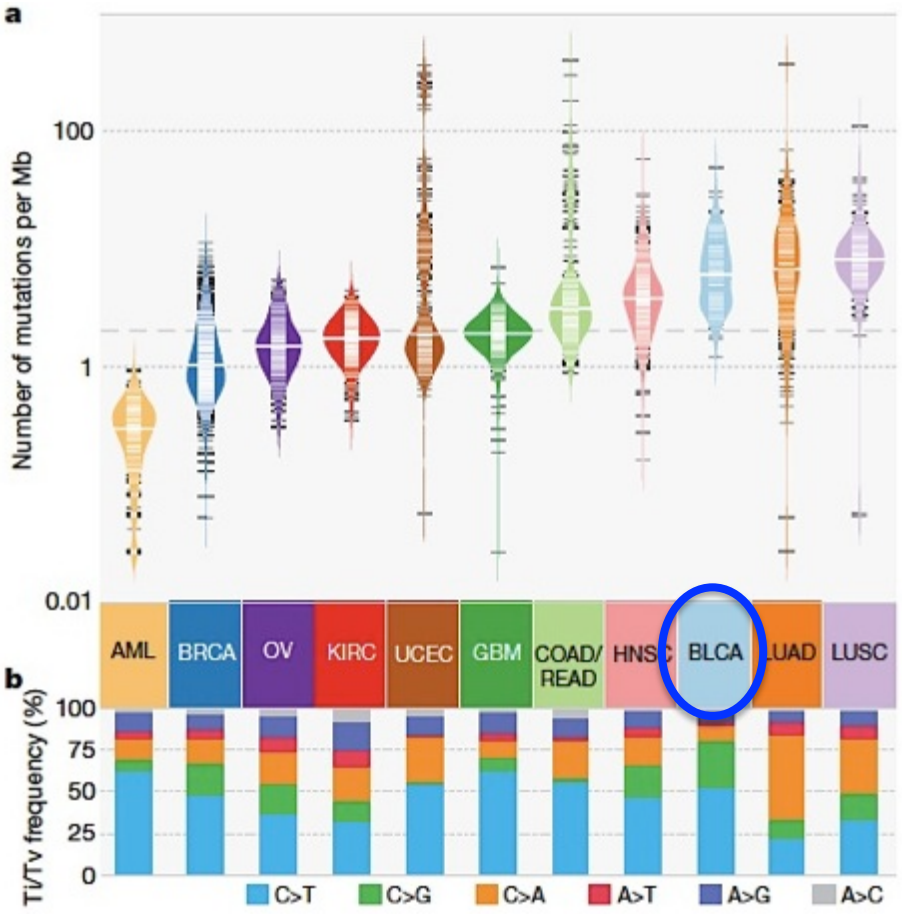
OPEN

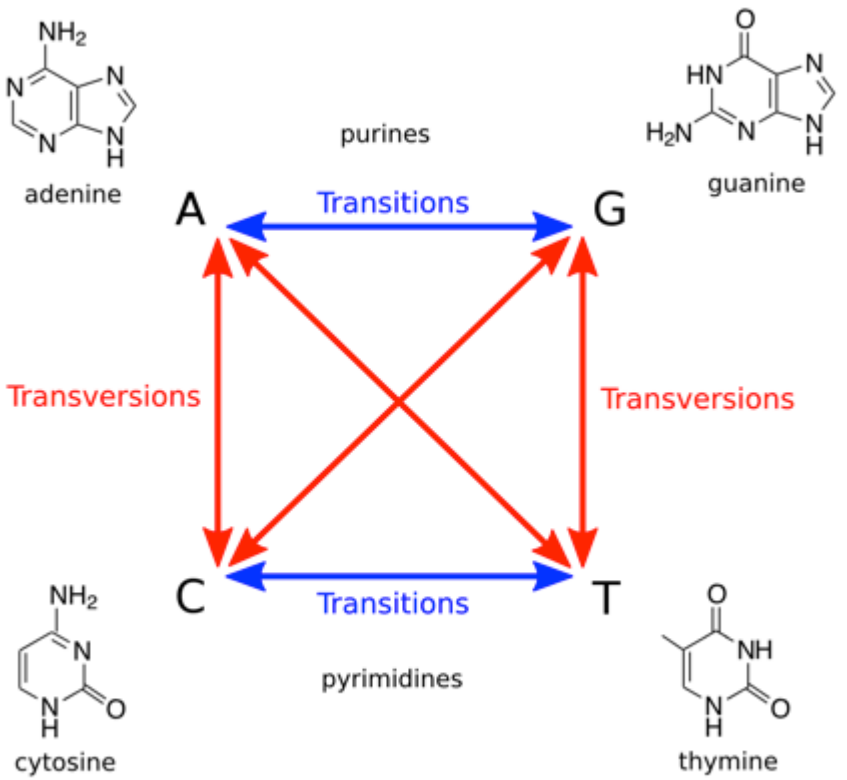
SUBJECT AREAS:
CANCER GENOMICS
COMPARATIVE GENOMICS

Exploring TCGA Pan-Cancer Data at the UCSC Cancer Genomics Browser

Melissa S. Cline, Brian Craft, Teresa Swatloski, Mary Goldman, Singer Ma, David Haussler & Jingchun Zhu

The molecular biology of Bladder Cancer



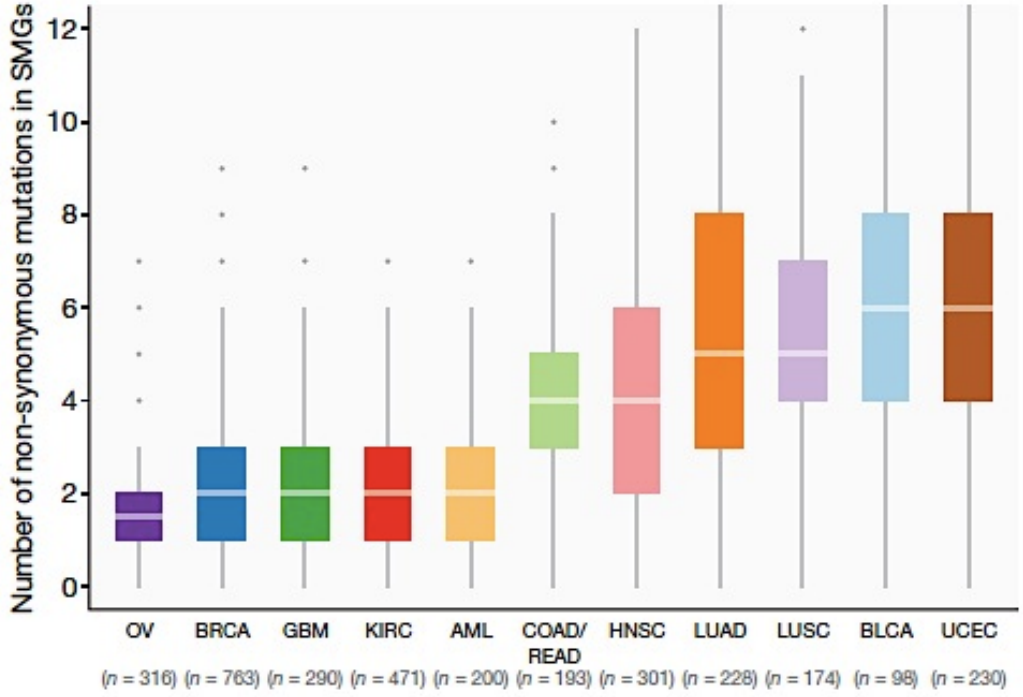


C>G transversions: oxidative stress
C>T transitions: abnormal methylation

C Kandoth *et al. Nature* **502**, 333-339 (2013) doi:10.1038/nature12634

The molecular biology of Bladder Cancer

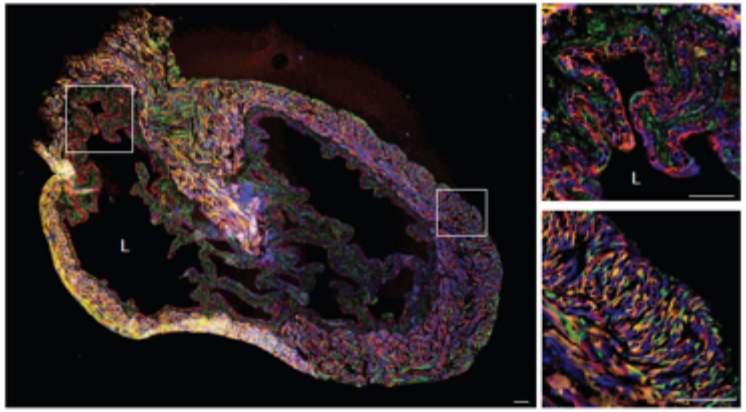
Distribution of mutations in 127 Significantly Mutated Genes (SMGs) across Pan-Cancer cohort.



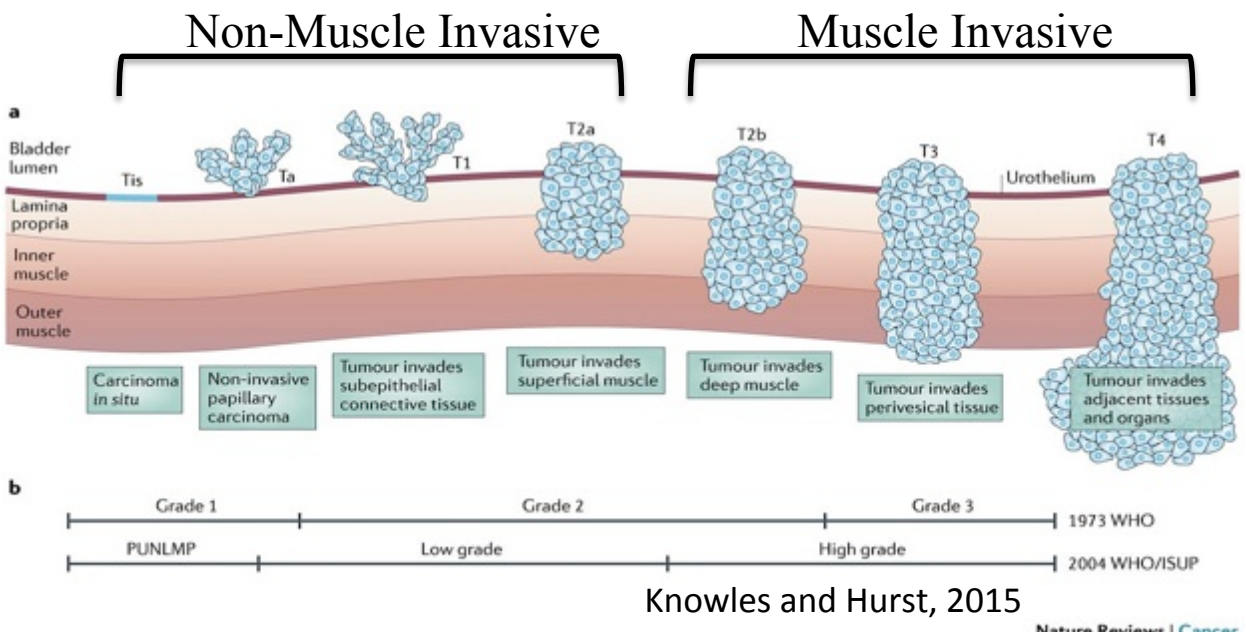
Box plot is the median number of non-synonymous mutations
3,210 tumors (hypermutators excluded) with 2-6 mutations/tumor
BLCA and UCEC (**Bladder and Uterine cancer the highest**)

C Kandoth *et al. Nature* **502**, 333-339 (2013) doi:10.1038/nature12634

The molecular biology of Bladder Cancer



Shin et al, Nature Cell Biology 2014



Knowles and Hurst, 2015

Genomic instability, chromosomal alterations and allelic loss in BLCA

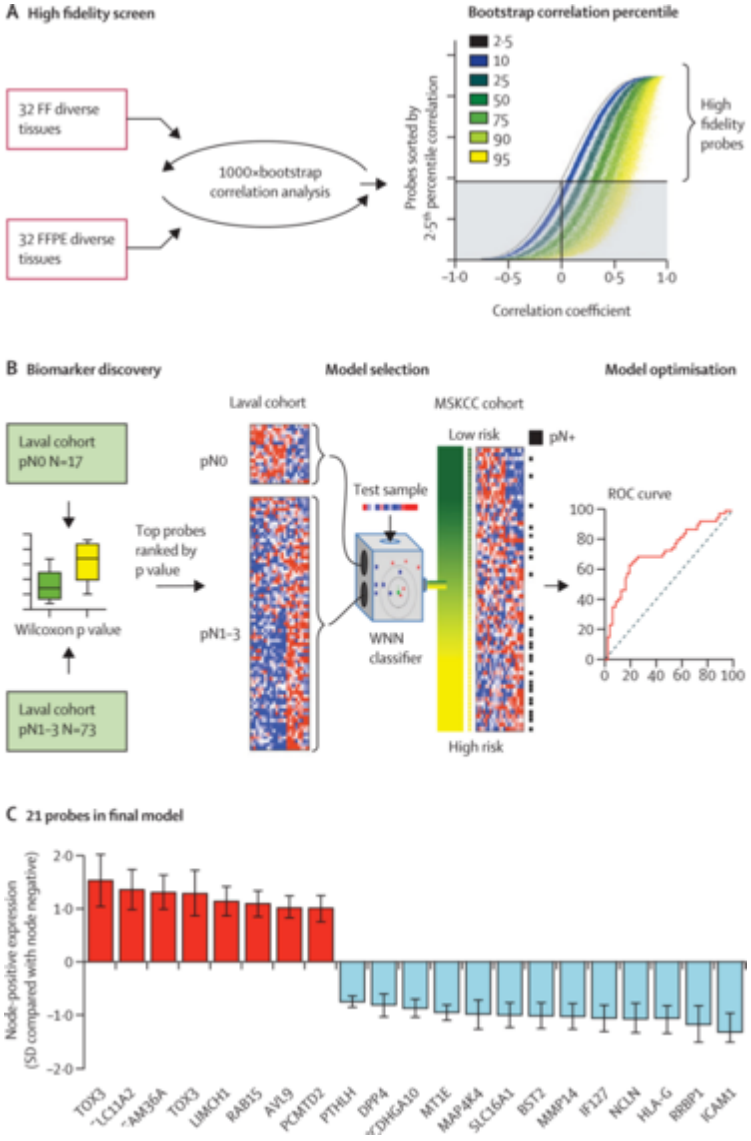
- Non-muscle invasive BC have near-diploid karyotype and few genomic rearrangements.

- Muscle-invasive BC commonly have
  - **Chromosome number changes: aneuploidy**
  - **Chromosomal alterations, translocations and chromothripsis**
  - **Non-homologous end-joining,** error-prone double-strand break repair
  - **Inactivating mutations**
    - DNA repair
    - DNA damage checkpoint genes
    - Chromatin and epigenetic modifiers: ARIDA1, CHD6, CREBBP, EP300, MLL1, 2 AND 3, NCOR1, KDM4, 6A

WHAT ARE WE MISSING ?

How do we study
Bladder Cancer?

20 microarray gene model for classification of risk in BLCA failed



In larger datasets, 20 genes

- Failed to identify specific markers
- Failed to identify common drivers

Smith et al, Lancet, 2011

nature genetics

ARTICLE

OPEN

doi:10.1038/nature12965

Comprehensive molecular characterization of urothelial bladder carcinoma

The Cancer Genome Atlas Research Network*

Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder

Yaoting Gui[1,12], Guangwu Guo[2,12], Yi Huang[1,12], Xueda Hu[2,12], Aifa Tang[1,3,12], Shengjie Gao[2], Renhua Wu[2],

nature genetics

Whole-genome sequencing identifies genomic heterogeneity at a nucleotide and chromosomal level in bladder cancer

Carl D. Morrison[a,1,2], Pengyuan Liu[b,1], Anna Woloszynska-Read[c], Jianmin Zhang[d], Wei Luo[c], Maochun Qin[e],

Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation

mors

Concurrent Alterations in *TERT*, *KDM6A*, and the BRCA Pathway in Bladder Cancer

Michael L. Nickerson[1], Garrett M. Dancik[2], Kate M. Im[1], Michael G. Edwards[3], Sevilay Turan[1], Joseph Brown[4], Christina Ruiz-Rodriguez[1], Charles Owens[2], James C. Costello[5], Guangwu Guo[6], Shirley X. Tsang[6], Yingrui Li[6], Quan Zhou[6], Zhiming Cai[7], Lee E. Moore[8], M. Scott Lucia[9], Michael Dean[1], and Dan Theodorescu[2,5,10]

Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity

Margaret A. Knowles and Carolyn D. Hurst

ARTICLE

OPEN

doi:10.1038/nature12965

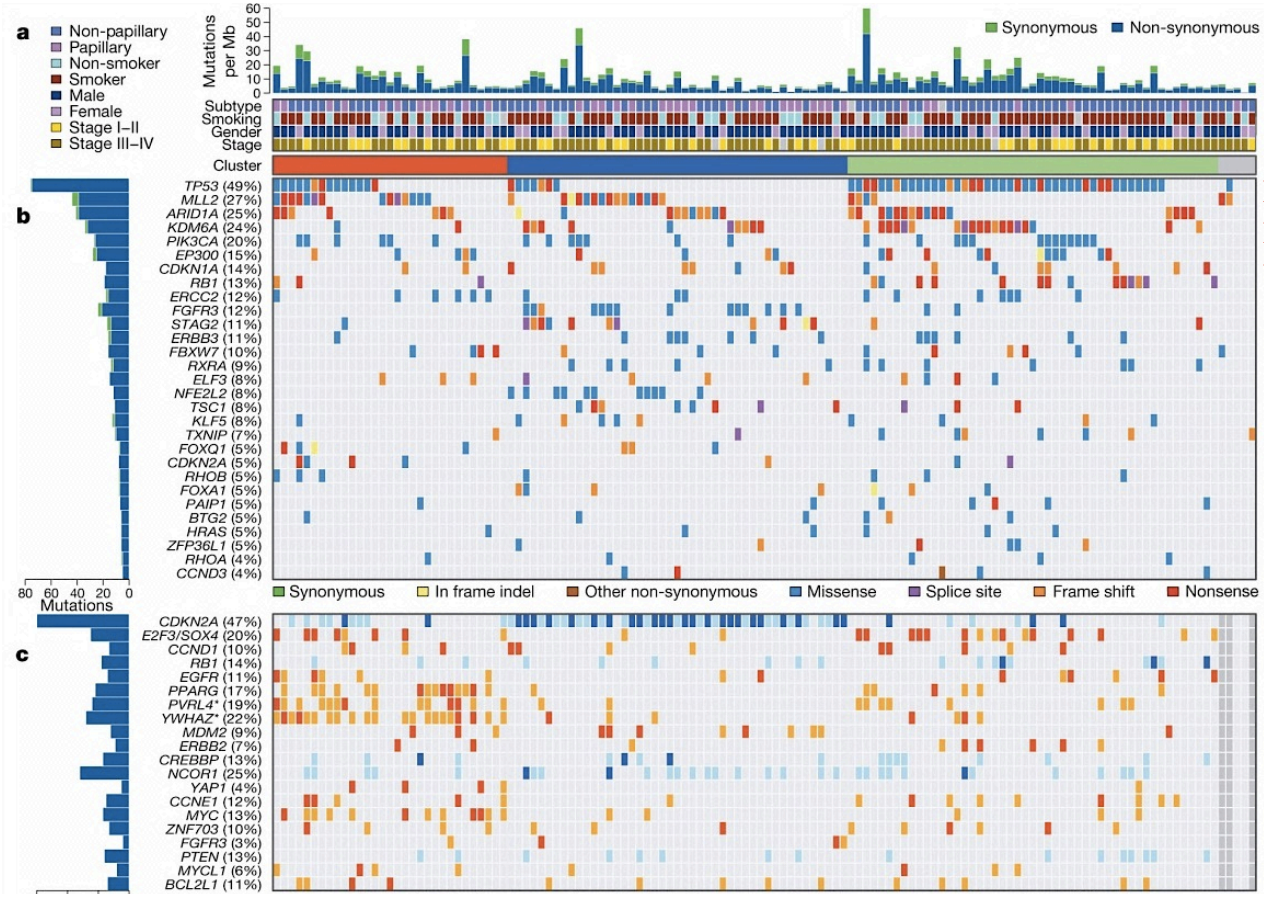
Comprehensive molecular characterization of urothelial bladder carcinoma

The Cancer Genome Atlas Research Network*

- 131 non-treated muscle invasive BLCA
- 186,260 exons and 18,091 genes
- Mean coverage of 100-fold, 82% target bases covered >30X.
- MuTect identified 39,312 somatic mutations
  - Mean and median somatic mutation rates of 5.5/ 1 Mb
- Average
  - 302 total mutations (slightly < than lung and melanoma)
  - 204 segmental alterations in genomic copy
  - 22 genomic rearrangements per sample
  - 27 amplified and 30 deleted recurrent somatic copy number alterations (CNAs)

TCGA Nature. 2014 Jan 29.

Genomic Landscape of Bladder Cancer



P53
MLL2, KDM6A,
ARID1A, EP300

Group Red, 'focally amplified', enriched in focal somatic CNAs includes ***chromatin remodelers***: MLL2, KDM6A, ARID1A, EP300;

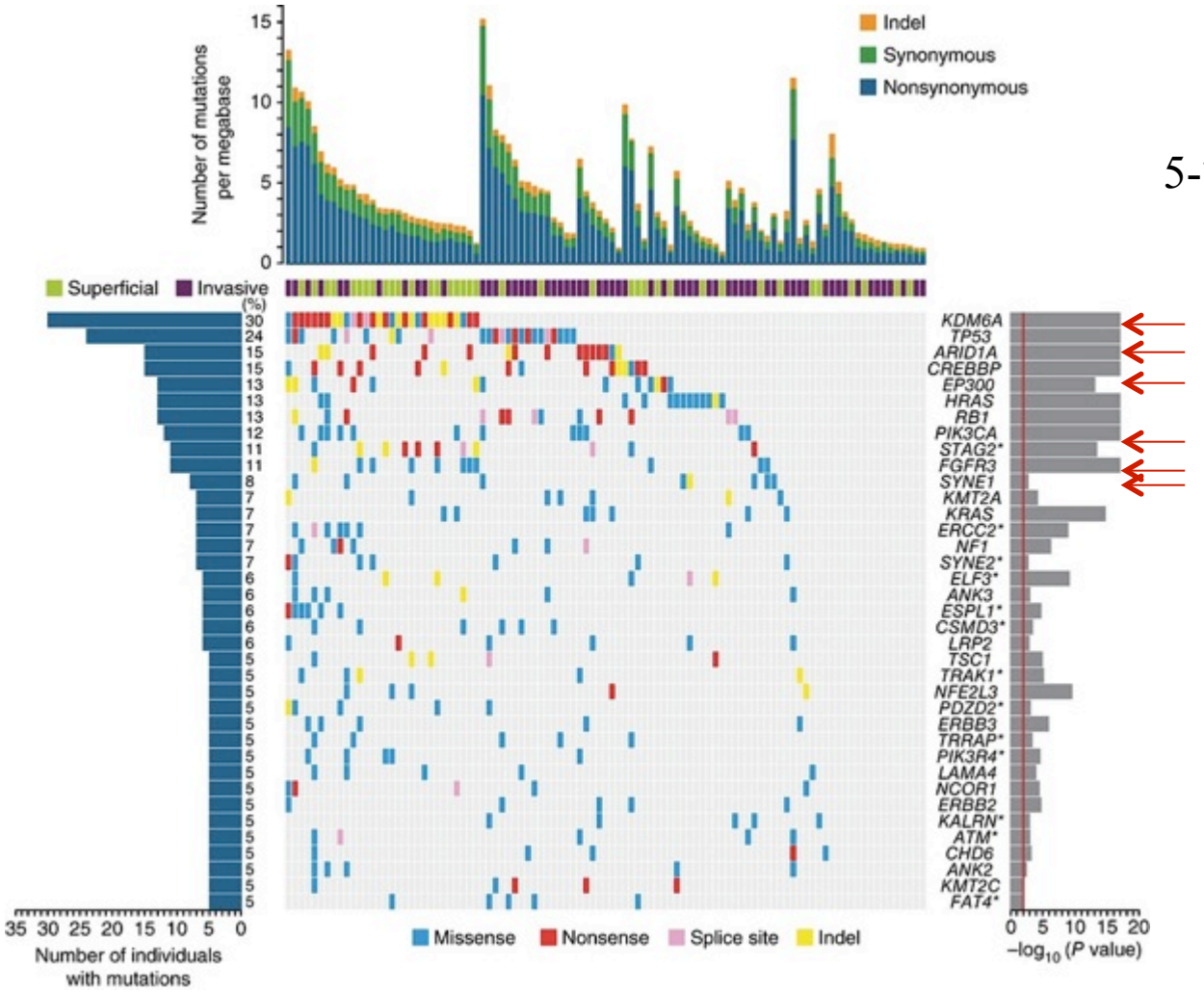
Blue: papillary, FGFR3 mutant, CDKN2A-deficient;

G**reen:** 'TP53/cell-cycle-mutant', RB1 mutations.

These differences in pattern suggest different oncogenic mechanisms.

TCGA Nature. 2014 Jan 29.

Genomic Landscape of Bladder Cancer



5-7.5 mutations/tumor

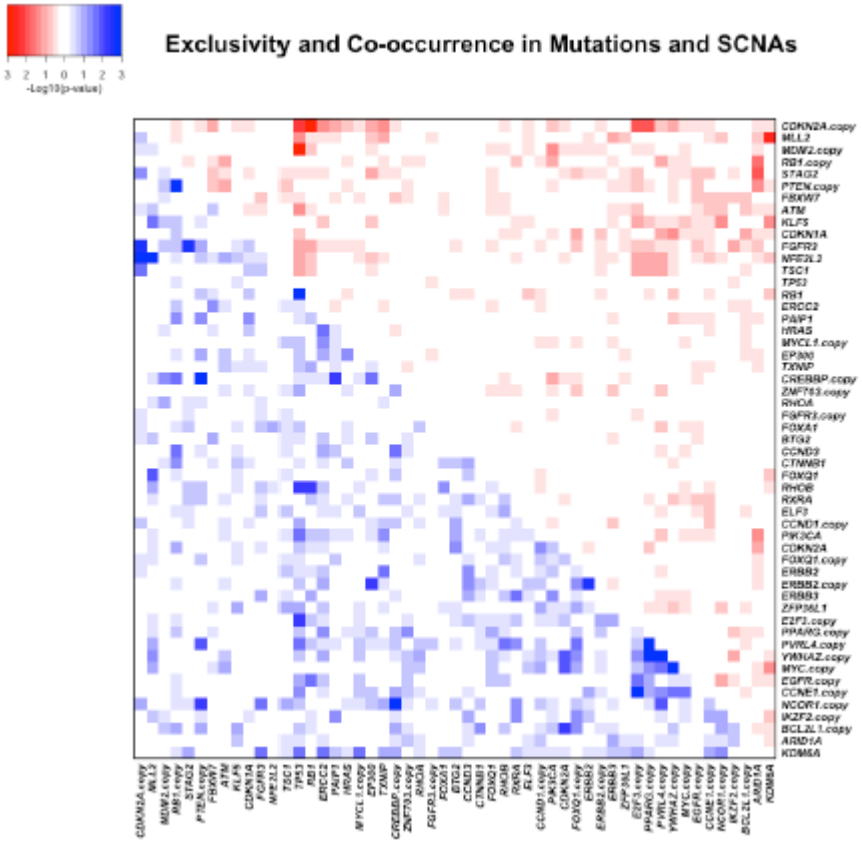
KDM6A, ARID1A, EP300
P53, RB1, HRAS, KRAS,
PI3KCA, FGFR3,
STAG2, SYNE2, ELF3

Guo, Nickerson at al, Nat Genet.45: 2013.

Genomic Landscape of Bladder Cancer

Mutual exclusivity correlations.

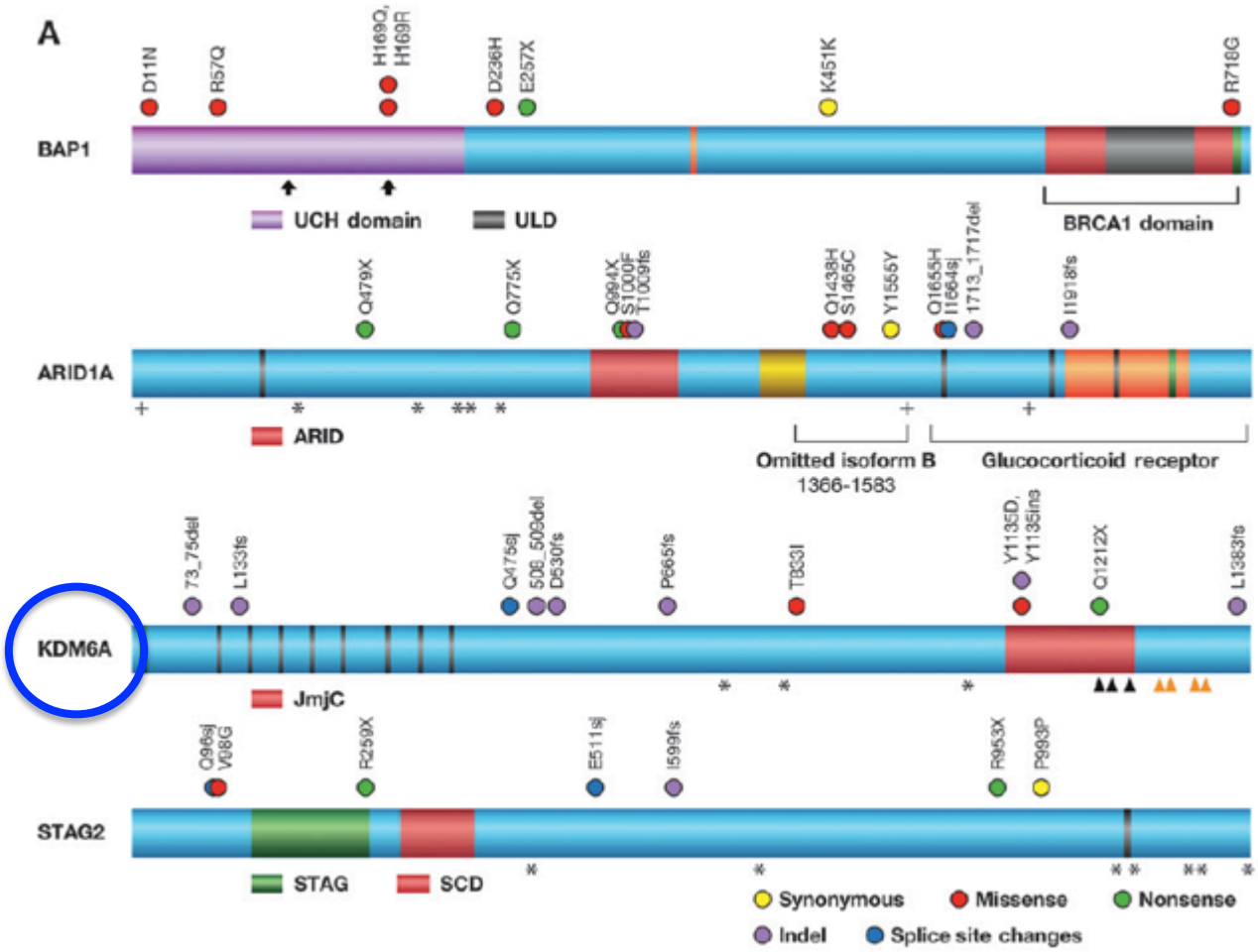
gene1	gene2	pval	qval
RB1	CDKN2A.copy	6.32E-06	0.00904392
TP53	MDM2.copy	0.000153	0.1094715
MLL2	KDM6A	0.00244	1
TP53	CDKN2A.copy	0.00477	1
CDKN2A.copy	PPARG.copy	0.00965	1
ARID1A	STAG2	0.0113	1
CDKN2A.copy	E2F3.copy	0.0129	1
ARID1A	RB1.copy	0.0241	1
ARID1A	PTEN.copy	0.0432	1
TXNIP	CDKN2A.copy	0.0438	1
KDM6A	MYC.copy	0.0443	1
ARID1A	PIK3CA	0.0456	1
KLF5	NCOR1.copy	0.0483	1
ERCC2	CDKN2A.copy	0.0488	1



- P53 + MDM2 ~80% of tumors
- MLL1,2 + KDM6A in ~ 70% of tumors

Guo, Nickerson at al, Nat Genet.45: 2013.

Mutation hot spots in BLCA



70% BRCA1 associated

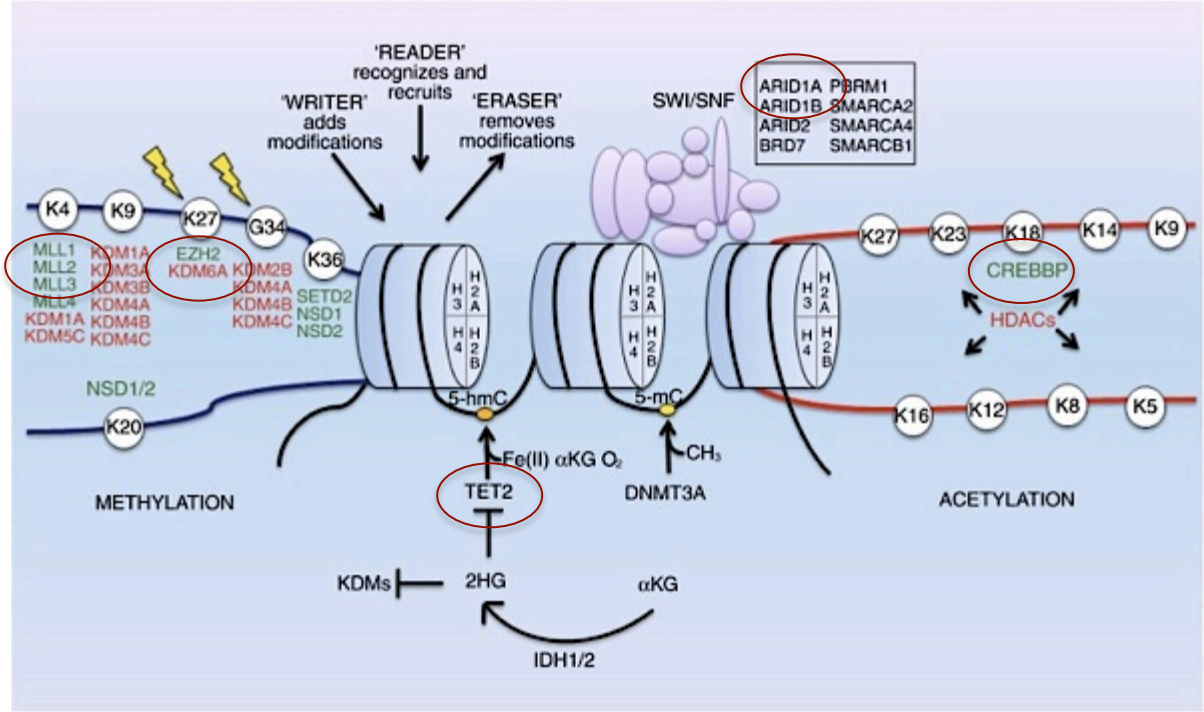
17% Chromatin remodeler

24% H3 K27 demethylase

17% Cohesin complex

Nickerson et al., ClinCaRes, 2014

Chromatin Modifiers



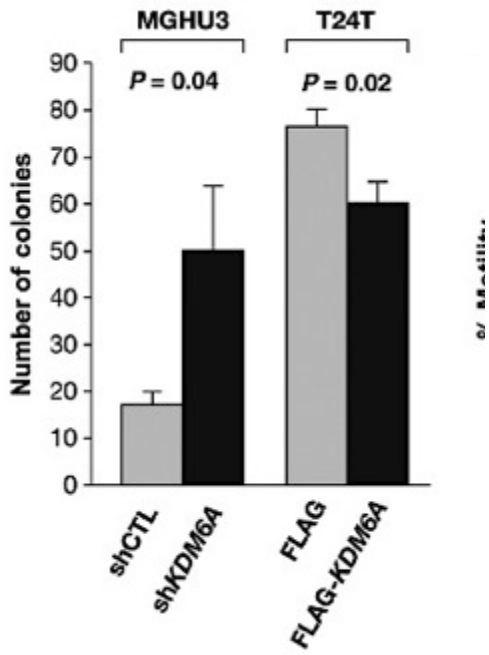
- DNA methylation
- Histone Modifications
- Chromatin remodeler
- Long noncoding RNAs
- microRNAs

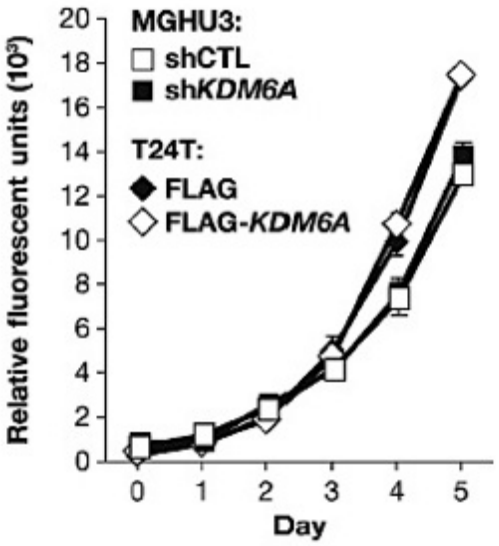
Is KDM6A a tumor suppressor in BLCA?

KDM6A histone 3 lysine 27 (H3K27) demethylase

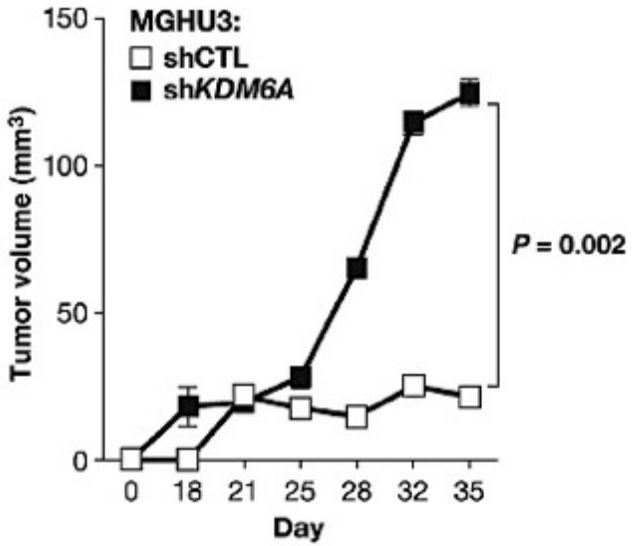
KDM6A KD increased and KI suppressed anchorage-independent growth

BLCA lines	KDM6A
MGHU3	+
T24T	-





In vitro: no change
In proliferation



In vivo: KDM6A KO
Enhanced tumor growth

Nickerson et al., ClinCaRes, 2014

SUMMARY-PART 1

Microarray analysis using gene expression did not identify common markers

Most frequent mutations are not in “driver” genes:

Muscle invasive BLCA have >5 mutations/tumor

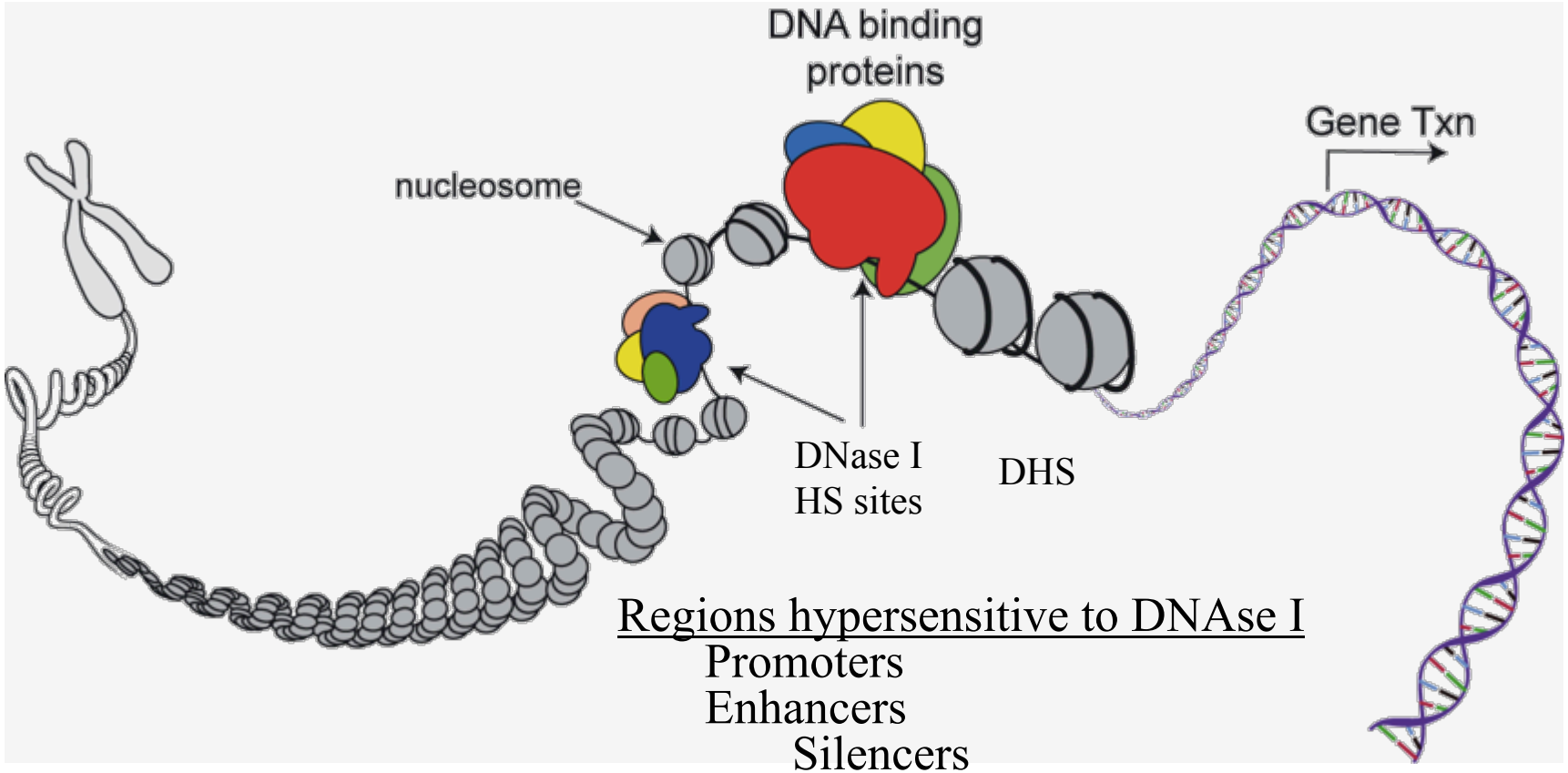
The most prominent group of genes after p53/RB1
are **Chromatin Modifiers:** KDM6A, MLL1,2,3, ARID1A, EP300, NCOA1

Chromatin Modifiers are mutually exclusive with MYC, P53, RB1, PI3KCA
suggesting overlapping functions

WHAT ARE WE MISSING ?

How do we study
chromatin modifications?

Genome-wide analysis of Chromatin Landscape by enzymatic digestion of intact chromatin: identification of DNAse I Hypersensitivity sites (DHS-seq)

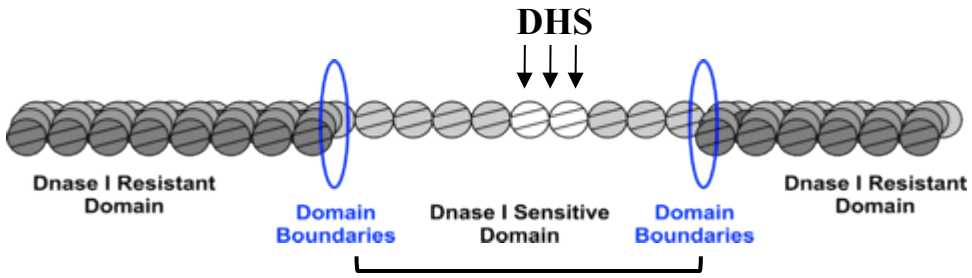


Regions hypersensitive to DNAse I

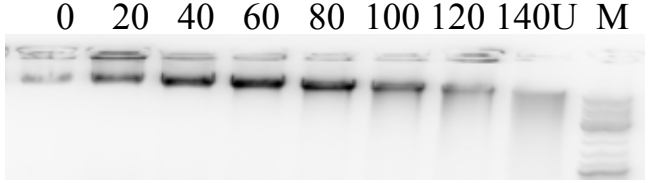
- Promoters
- Enhancers
- Silencers
- Insulators
- Locus control regions

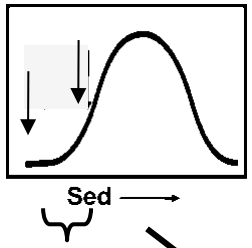
Each cell type will have a unique landscape signature

Genome-wide analysis of Chromatin Landscape by enzymatic digestion of intact chromatin: identification of DNAse I Hypersensitivity sites (DHS-seq)



Separate fragments on sucrose gradient

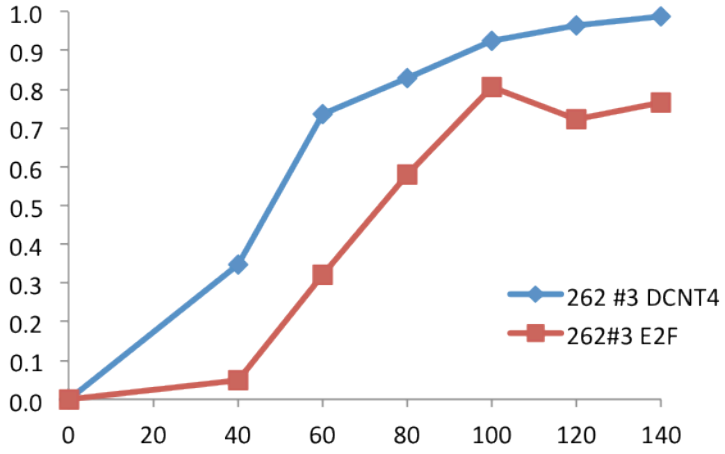




DNase-Seq

Massive parallel sequencing

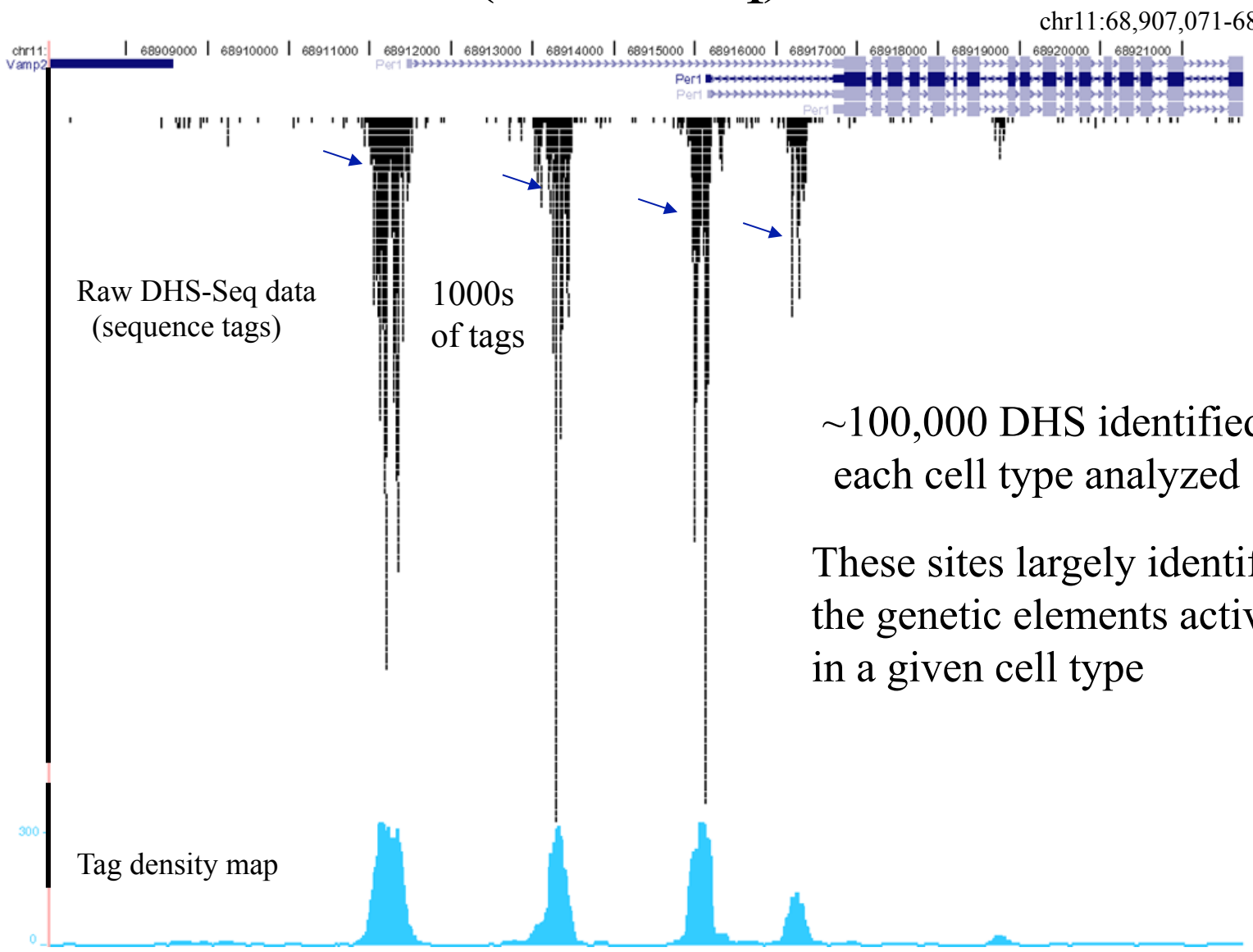
Align to the genome
Bioinformatics
Compare to ChIP-seq (if needed)



DNase-I U/ml

Nature Genetics (2011)

Genome-wide mapping DNAse I hypersensitive sites (DHS-Seq)

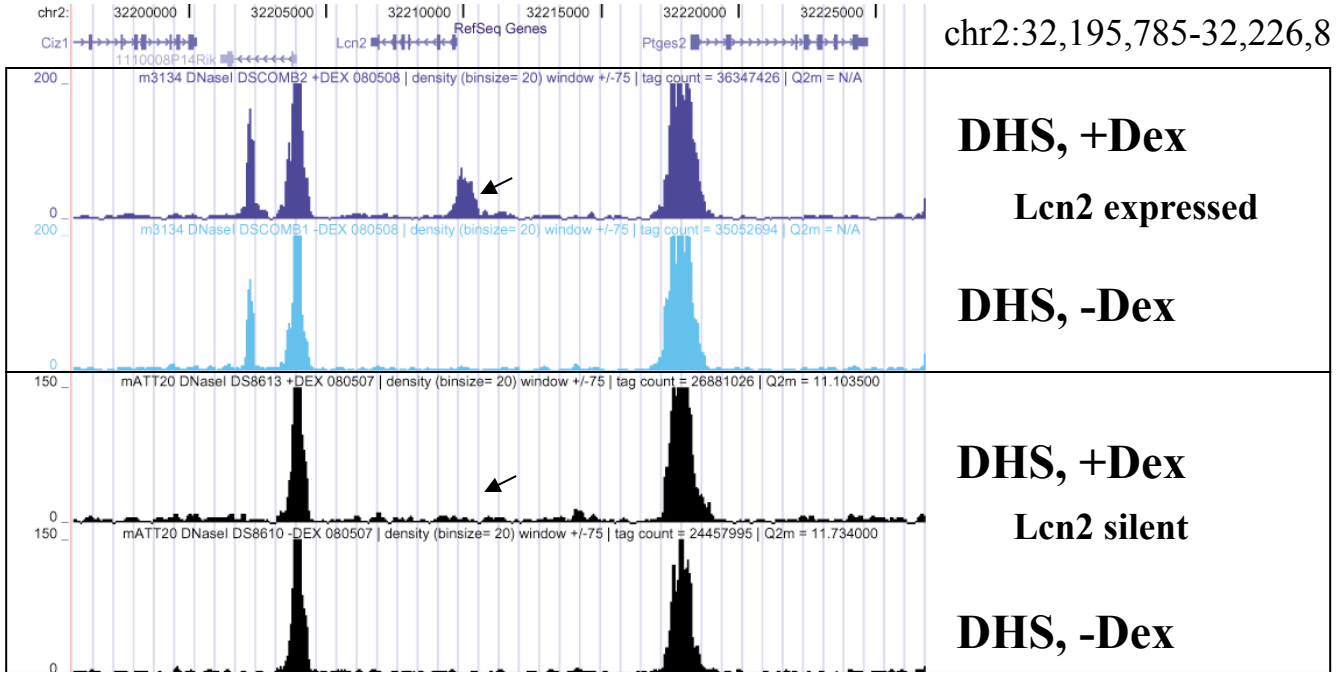


Cell Specific Chromatin Structures

Lcn2
Active only in mammary cell

3134 Mammary Cell Line

AtT-20 Pituitary Cell Line



chr2:32,195,785-32,226,814

DHS, +Dex

Lcn2 expressed

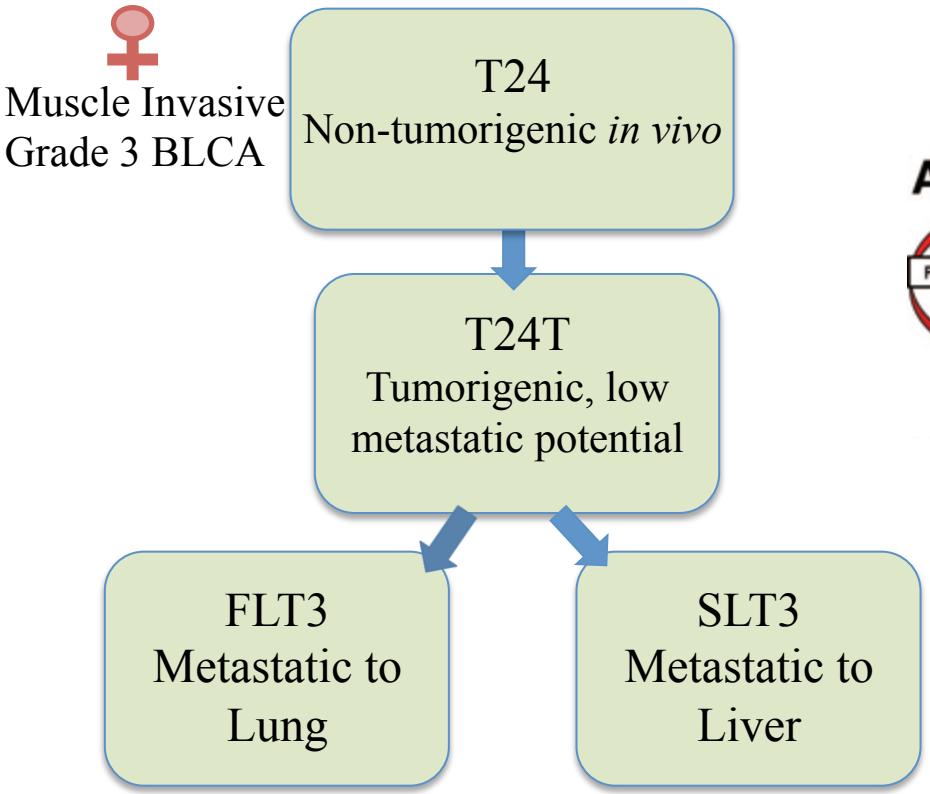
DHS, -Dex

DHS, +Dex

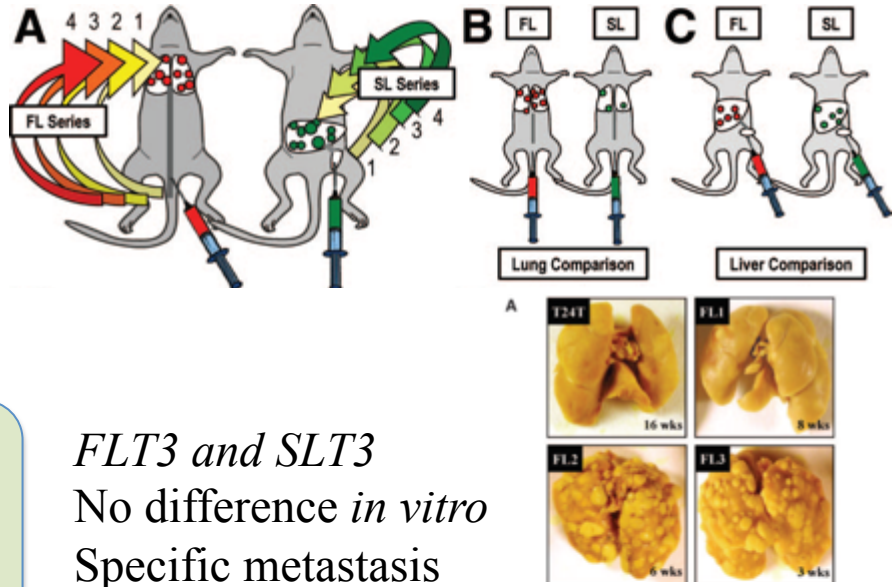
Lcn2 silent

DHS, -Dex

Tumor progression analysis by DHS-seq



T24T selected in vivo for metastasis to lung and liver



FLT3 and SLT3
No difference *in vitro*
Specific metastasis

Bladder cell lines selected *in vivo* allow us to understand the changes in DHS landscape during tumor progression and metastasis.

Dan Theodorescu, U. CO

Exon sequencing mutations

Common	Mutations	T24	T24T	FL3	SLT3
AHNAK2	p.A1342ins FS				
AOAH	p.M659ins FS				
AOAH	p.P639ins FS				
AQP7	p.Q30_R31delinsRGRX				
DHDH	p.A170ins FS				
DHDH	p.294_294del FS				
DNAH17	p.I1311V				
EP300	p.C1201Y	+L	+L	+L	+L
EP400	p.Q2726delinsQQQQ NFS				
FAT4	p.D2672V				
FGFR3	p.IVS-2				
HMCN1	p.E5601K				
HRAS	c.G35T				
KDM6A	p.E895X	+L	+L	+L	+L
MLL2	p.P692T	+L	+L	+L	+L
MLL3	p.S772L				
MLL3	p.P2412T				
MS4A14	p.56_56del FS				
RELN	p.D2171G	+L	+L	+L	+L
TP53	p.Y126X	+L	+L	+L	+L
CDKN2B		HL	HL	HL	HL

Subclonal					
EP400	p.P581delinsM NFS				
EP400	p.V1156I				
NCOA1	p.R1122X				
DNMT1	p.V1367L				
ANK3	p.A2700S				
RELN	p.G1612V				

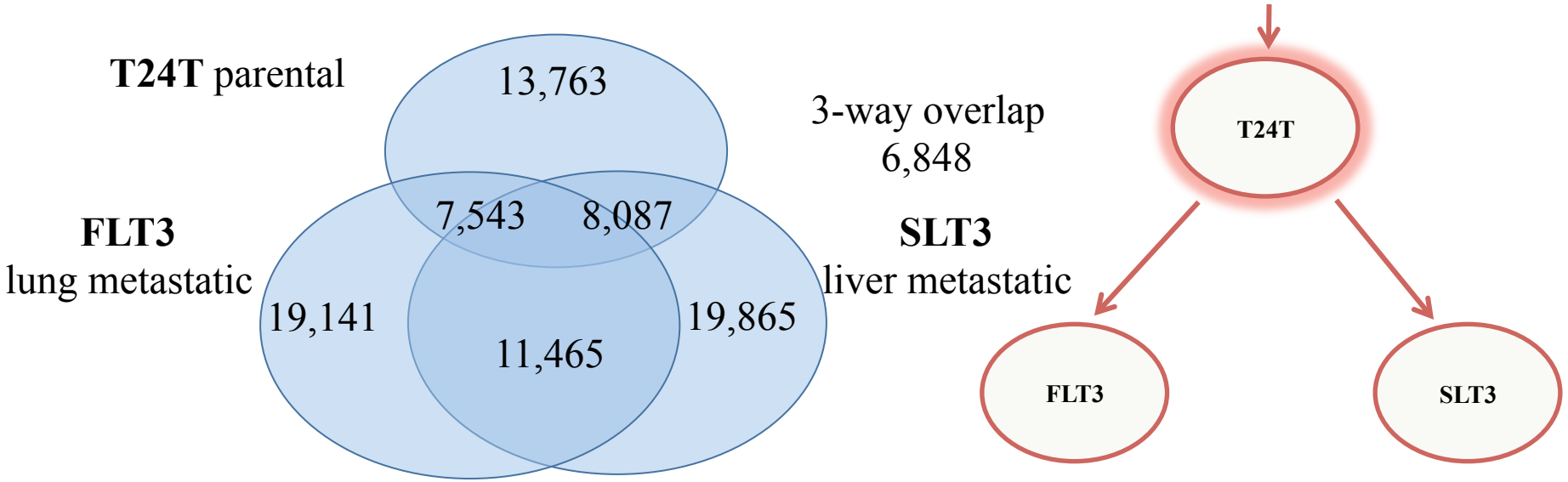
Grey, variants, selected for those that alter proteins

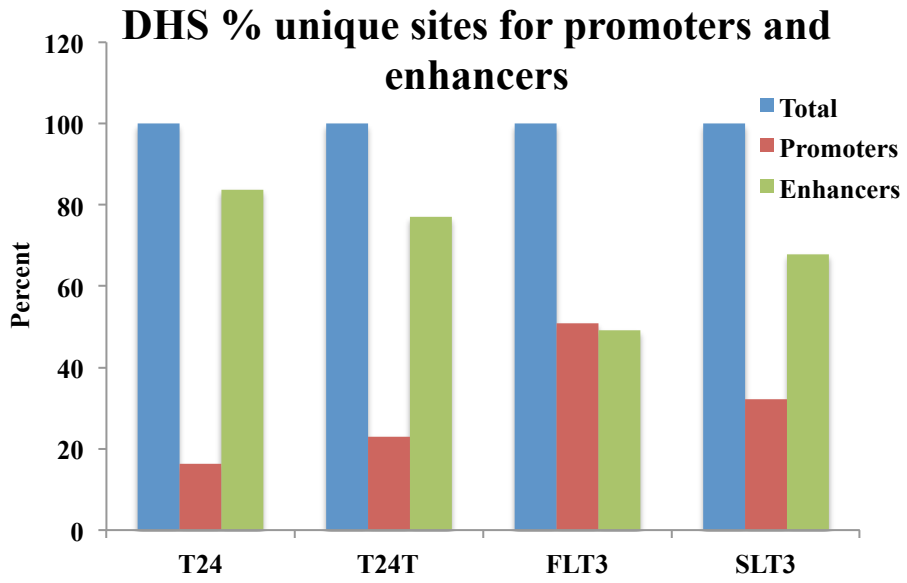
+L, Loss of heterozygosity (LOH) of the WT allele in addition to the indicated sequence alteration

HL, homozygous loss indicated by no NGS reads

Nickerson, NCI and Theodorescu, U. CO

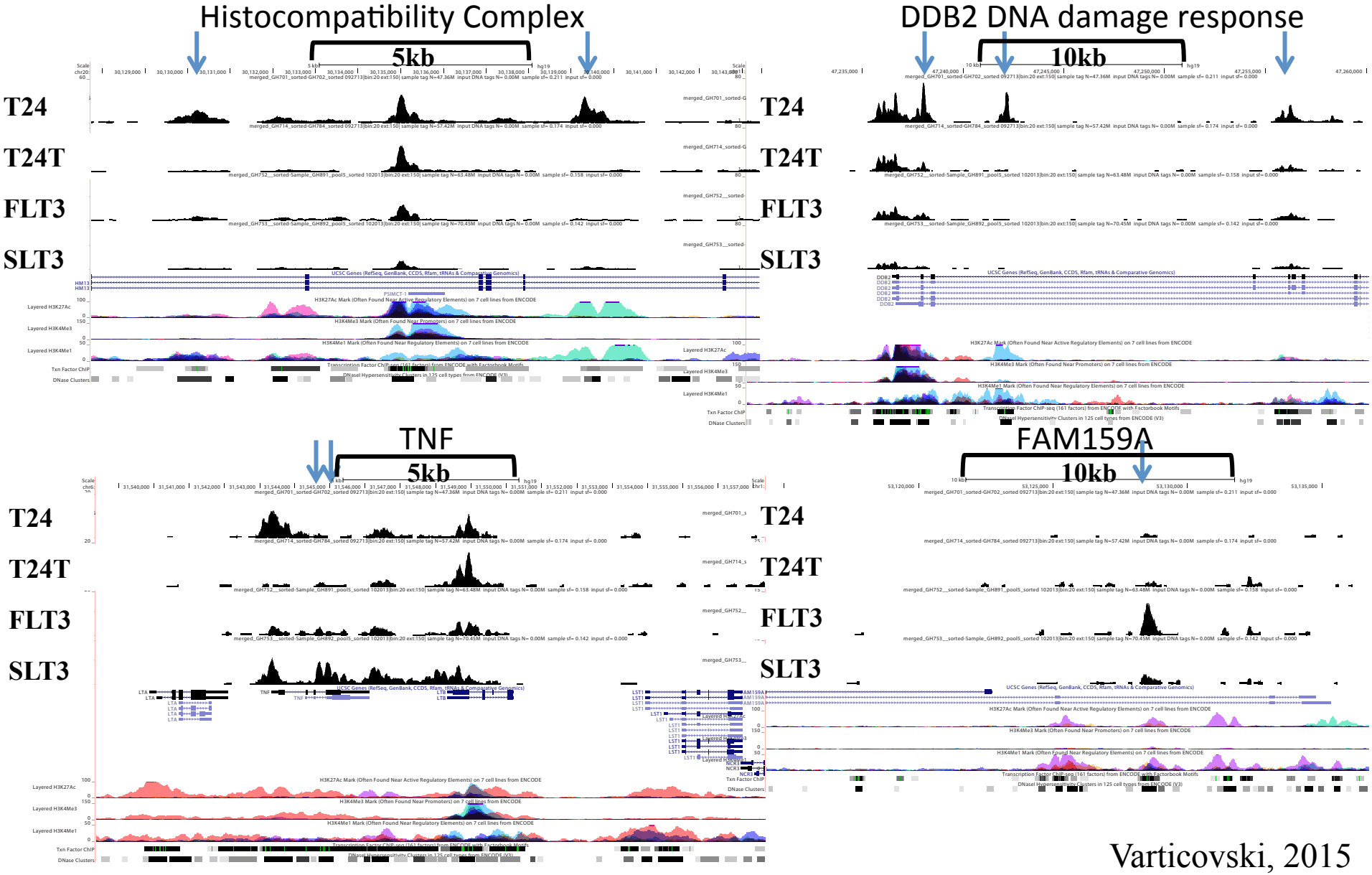
Genome-wide analysis of Chromatin Landscape by DNAse I Hypersensitivity sites (DHS-seq)





Varticovski, 2015

Genome-wide analysis of Chromatin Landscape by DHS-seq



Varticovski, 2015

Is DHS-seq on BLCA comparable to the gene expression by microarray?

Tools:

DHS-seq cell lines
Microarray analysis at similar growth characteristics

Procedure:

1. Analyze Microarray by Ingenuity pathway (IPA)
   using all known published data
1. Analyze each cell type unique genes within 50kb of DHS
2. Build overlapping and unique pathways for each type of analysis.

Identification of biological pathways in progression to metastatic phenotype by analysis of changes in global chromatin landscape

Merging DNAse I hypersensitivity and microarray using Ingenuity Pathway Analysis (IPA)

- Analyze Microarray by Ingenuity pathway (IPA) using all known published data
- Analyze genes within 50kb of each DHS, unique for each cell
- Build pathways DHS and overlap with gene expression

Conclusions

- Chromatin remodeling enzymes emerged as a major group of genes involved in cancers
  - specifically in those that lack the known “driver” mutations
- DNAse I hypersensitivity (DHS-seq) permits analysis of unbiased chromatin landscape
  - Specifically useful in analysis of tumor progression
  - Can identify specific TF binding motifs
  - Drug response?
- DHS-seq could provide specific signature of tumor type
  - Diagnostic/staging potential
- BLCA cell lines with metastatic progression
  - Unbiased analysis of DHS in enhancers identified metastatic site
  - Network genes involved by DHS correlate with expression data

Thank you

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Diego Presman
Lou Schiltz
Diana Stavreva
Mia Sung
Erin Swinstead
Bethtrice Thompson
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NCI, CCR
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